Product Datasheet

Anti-Glypican 1 (GPC1) [IPI-GPC1.25]

Applications | Antibody Details | Antigen Details | References

Overview

Antigen	Glypican 1 (GPC1)
Immunogen	Purified recombinant fragment of Human Glypican 1 (GPC1), corresponding to AA: 24-498.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-GPC1.25
RRID	AB_3697521
IPI ID	TAB0010734-013-002
Specificity	GPC1; Does not recognize other GPCs
Species reactivity	Human and mouse
Amount	100 µg
Concentration	1 mg/mL
Purification	Expressed in HEK293 cells and affinity purified using Protein A
Storage buffer	PBS, pH 7.4
Shipping	Shipped on blue ice at +4C
Storage	Store at +4C for up to 3 months. For long-term storage, aliquot and store at -20C. Avoid multiple freeze/thaw cycles.

IPI Tested Applications‡

Application	Tested concentration	Result	Reference
Flow	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.i5a2vk
IF – Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.i20e4x
IF – Specificity	1 µg/mL	Positive	https://doi.org/10.57733/addgene.q3ed6a
IF – Endogenous	5 µg/mL	Positive	https://doi.org/10.57733/addgene.mclt3n

‡ Not suitable for WB application.

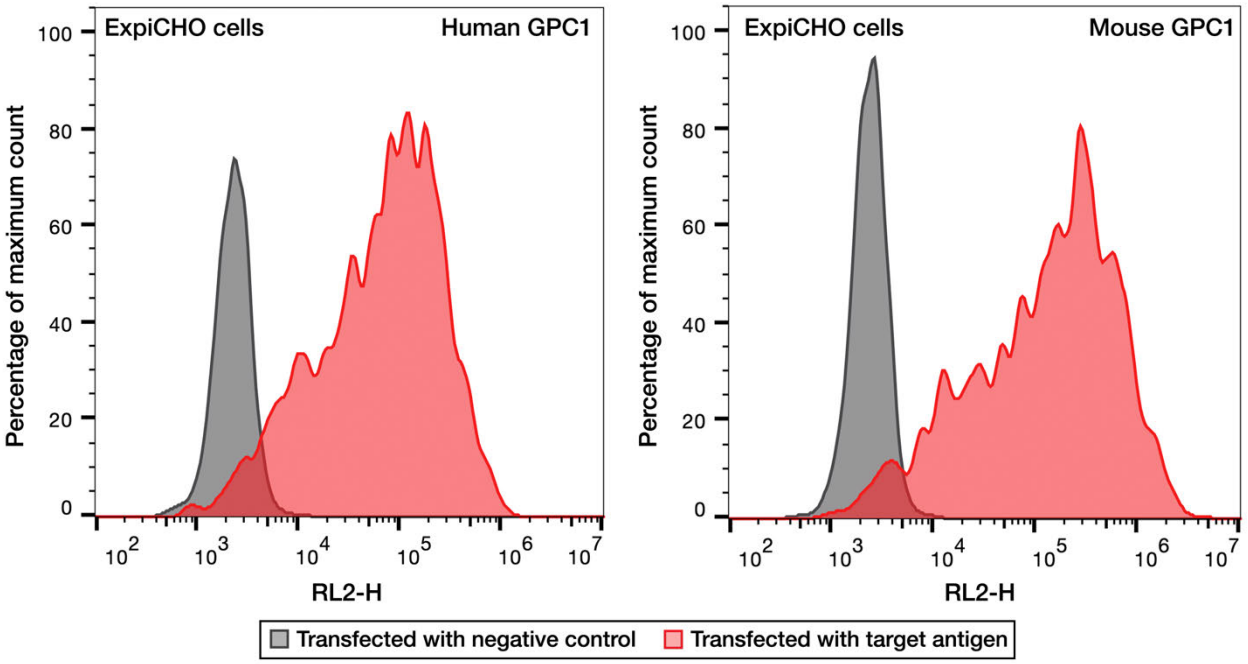
Community Data*

Application	Lab	Reference
IHC	James Trimmer, Ph.D., UC Davis/NeuroMab	https://doi.org/10.57733/addgene.7d1701

* Supporting Data is generated by external partner labs, in the process of evaluating IPI antibody panels.

Applications

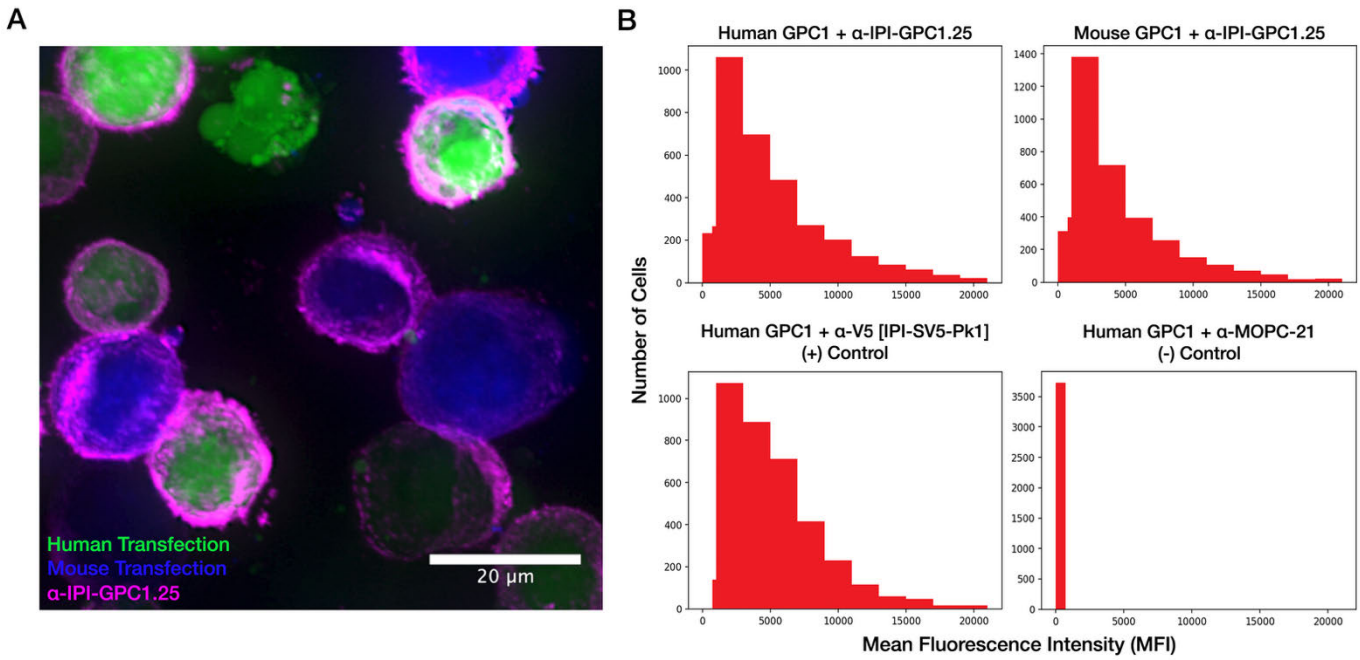
Flow cytometry



Anti-Glypican 1 (GPC1) [IPI-GPC1.25] (Addgene #240996) recognizes human and mouse GPC1 in flow cytometry. Histograms from FACS analysis on ExpiCHO cells transfected with human or mouse GPC1 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) GPC1 were labeled with Anti-Glypican 1 (GPC1) [IPI-GPC1.25] (15 μg/mL) and Alexa Fluor 647 F(ab')2 goat anti-rabbit IgG Fc fragment (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Histograms were generated and normalized to mode using FlowJo™ v10.10. doi: https://doi.org/10.57733/addgene.i5a2vk

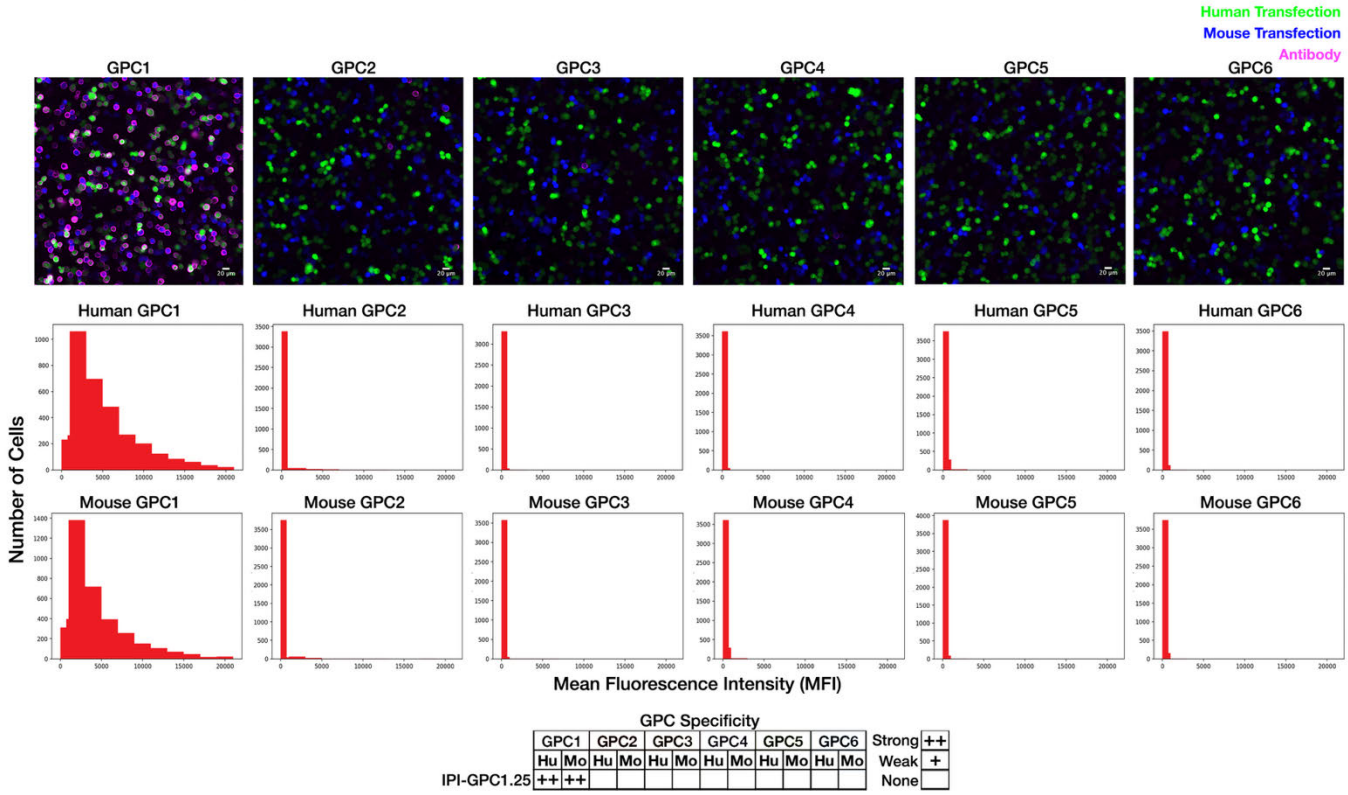
EC_{50} (data not shown): A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse GPC1 showed reactivity towards human and mouse GPC1 with observed EC_{50} values of 4.45 nM and 3.82 nM for human and mouse GPC1, respectively.

Immunofluorescence (IF) – Species Reactivity



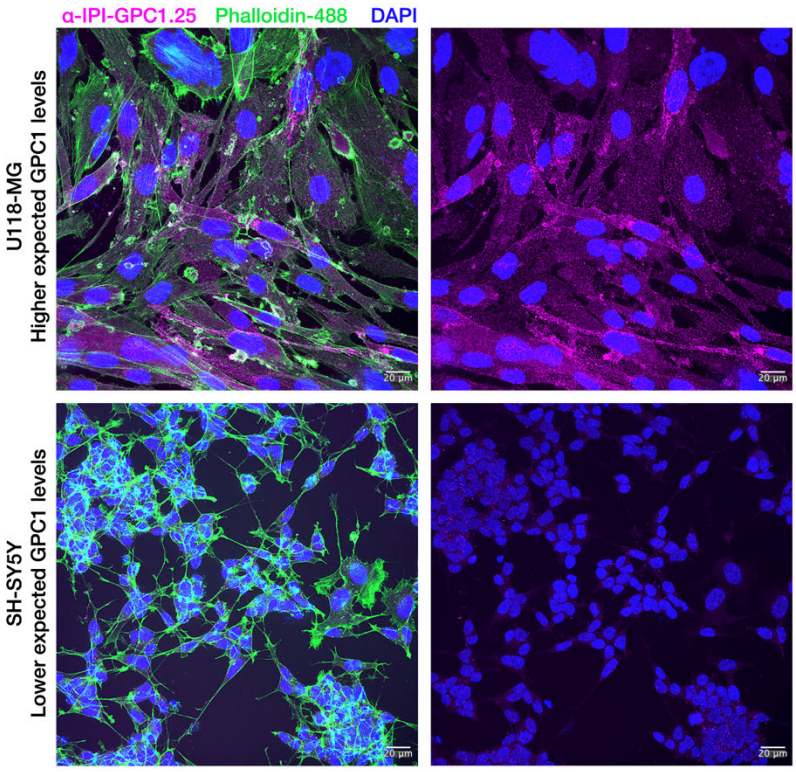
Anti-Glypican 1 (GPC1) [IPI-GPC1.25] (Addgene #240996) recognizes human and mouse GPC1 in immunofluorescence. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human and mouse GPC1. Human GPC1 was co-transfected with GFP (human transfection control) and mouse GPC1 was co-transfected with BFP (mouse transfection control). Confocal images taken at 40X magnification on the ImageXpress confocal HT.ai microscope. Cells were imaged for GFP (green), BFP (blue), and GPC1 (magenta). B) Combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP- or BFP- positive cells were identified using a custom module in the IN Carta image analysis software, then the mean fluorescence intensity (MFI) of the far-red channel for each cell, representing IPI-GPC1.25 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 450 (background fluorescence) to 20000. IPI-GPC1.25 staining of human (left) and mouse (right) GPC1 are shown in the top row, and compared to a positive (left) and negative (right) controls in the bottom row. For both panels, IPI-GPC1.25 was used at 1 µg/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.i20e4x

Immunofluorescence (IF) – Target Specificity



Anti-Glypican 1 (GPC1) [IPI-GPC1.25] (Addgene #240996) is specific for GPC1 in immunofluorescence. (Top) Immunofluorescence (IF) of ExpiCHO cells transfected with human and mouse GPC1-GPC6. Human GPC targets were co-transfected with GFP (human transfection control) and mouse GPC targets were co-transfected with BFP (mouse transfection control). Widefield images were taken at 10X magnification on an imageXpress confocal HT.ai microscope. (Bottom) Each graph depicts the combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP- or BFP- positive cells were identified using a custom module in the InCarta software, then the mean fluorescence intensity (MFI) of the far-red channel for each cell, representing IPI-GPC1.25 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 450 (background fluorescence) to 20000. IPI-GPC1.25 staining of human and mouse variants of each GPC family member is compared on the top and bottom rows. For all panels, IPI-GPC1.25 was used at 1 µg/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.q3ed6a

Immunofluorescence (IF) – Endogenous



Anti-Glypican 1 (GPC1) [IPI-GPC1.25] (Addgene #240996) recognizes endogenous GPC1 in immunofluorescence. Immunofluorescence (IF) of U118-MG cells (top row) and SH-SY-5Y cells (bottom row) stained for GPC1 using IPI-GPC1.25 and for actin with Alexa Fluor 488-conjugated phalloidin. Confocal images taken at 40X magnification on an ImageXpress HT confocal.ai microscope. Left images show all 3 channels (IPI-GPC1.25, actin, and DAPI) while the right images only show IPI-GPC1.25 and DAPI. U118-MG cells are predicted to express GPC1 highly and SH-SY-5Y cells are predicted to not express GPC1 highly based on publicly available data (DepMap.org). For both panels, IPI-GPC1.25 was used at 5 µg/mL (1:200 dilution). doi: https://doi.org/10.57733/addgene.mclt3n

Antibody Details

Antibody design and production

Human variable domains for the heavy and light chain of the FAB fragment used in yeast display were grafted onto the constant CH1, CH2 and CH3 domains of rabbit IgG. The chimera human/rabbit IgG1 construct was recombinantly expressed in Expi HEK293 cells, using pTipi2.1 as the expression vector. The antibody was purified by affinity chromatography using protein A (XYZ) and acid elution, followed by immediate buffer exchange using 1 x PBS buffer pH 7.4.

Sequence information

Heavy chain and light chain amino acid sequences are available upon request after purchase. **Contact us** to request.

Antibody Characterization

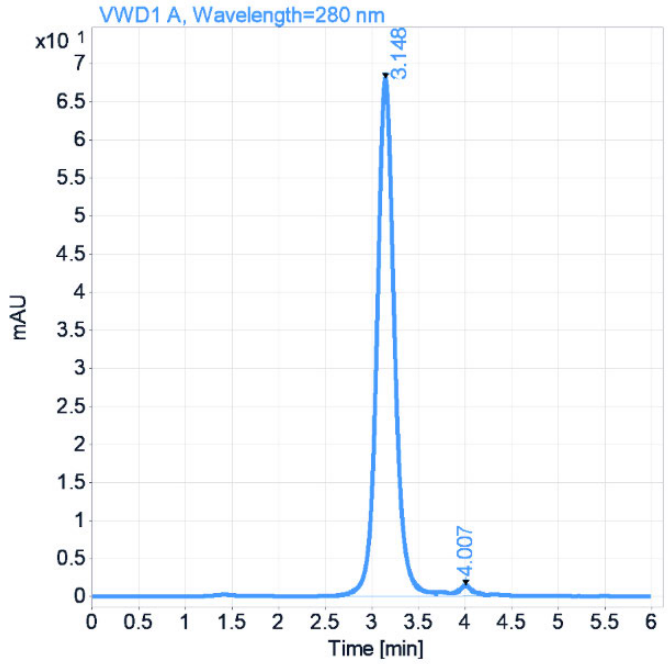
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation of the masses corresponding to the amino acid sequences of both chains.

	HC MW (Da) *Calculated*	HC MW (Da) *Observed*	HC MW (Da) Delta	LC MW (Da) *Calculated*	LC MW (Da) *Observed*	LC MW (Da) Delta
IPI-GPC1.25	50403.34	50407.00	3.66	22944.51	22944.32	-0.19

Heavy Chain (HC) Mass Calculation: The calculated molecular weight (MW) of the HC is derived by adding the mass of the unmodified HC amino acid sequence to the mass of the predominant N-glycan form (G0F), which is 1444.5 Da. This calculation assumes that the intrachain disulfide bonds remain intact. For HCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), resulting in a deduction of 17.03 Da from the total mass. Additionally, for HCs with a C-terminal lysine (K), the mass of K (128.09 Da) is also subtracted.

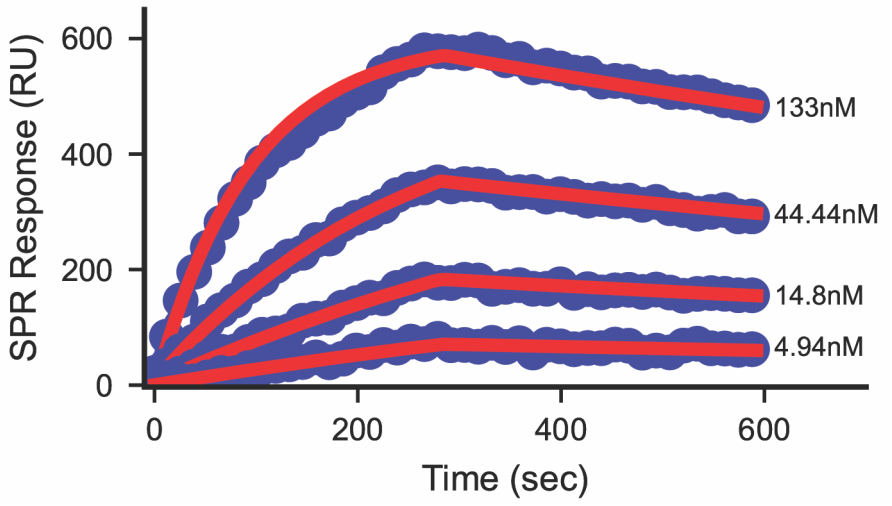
Light Chain (LC) Mass Calculation: The calculated molecular weight (MW) of the LC is obtained from the mass of the unmodified LC amino acid sequence, assuming that the intrachain disulfide bonds are not reduced. For LCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), leading to a deduction of 17.03 Da from the total mass. For LCs with a C-terminal lysine (K), the mass of K (128.09 Da) is subtracted as well.

Size Exclusion Chromatography (SEC): SEC is a protein purification technique that separates molecules based on size.



	RT (min)	Width (min)	Area	Height	Area %	Result
IPI-GPC1.25	3.148	0.2189	893.1	68.0032	97.8285	Pass

Surface Plasmon Resonance (SPR)



	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Res. sd
IPI-GPC1.25	7.6×10^{4}	5.5×10^{-4}	7.2×10^{-9}	9.2

Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-GPC1 [IPI-GPC1.25] and human GPC1. SPR binding kinetics were measured on a Carterra LSA using HC30M chips (Carterra, cat. #4279) at 25 °C. Goat anti-rabbit IgG Fc (Jackson ImmunoResearch, cat. #111-005-046) was immobilized via amine coupling, and test antibodies were captured using a 96-channel print-head. Antigens (400 nM to five lower concentrations, 2-fold dilutions) were injected in antigen buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.005% Tween 80) with 300 s association/dissociation phases and acid regeneration. Data (reference/buffer subtracted, smoothed) were globally fit to a 1:1 Langmuir model to derive k_a, k_d, and K_D using Carterra Kinetics software v1.9.2.44.63, and replotted in OriginPro 2023b. Results show a high-affinity and specific binding event between the antibody and antigen.

Antigen Details

Immunogen design:

cDNA of Human Glypican 1 (GPC1) with C-terminal His-, FLAG-, Avi-, and HA-tags was produced in transiently transfected Expi293F cells and purified from culture supernatant by Ni-NTA affinity purification followed by size-exclusion chromatography.

Immunogen sequences:

>Human GPC1 (AA: 24-498):
DPASKSRSCGEVRQIYGAKGFSLSDVPQAEISGEHLRICPQGYTCCTSEMEENLANRSHAELETALRDSS
RVLQAMLATQLRSFDDHFQHLLNDSERTLQATFPGAFGELYTQNARAFRDLYSELRLYYRGANLHLEETL
AEFWARLLERLFKQLHPQLLLPDDYLDCLGKQAEALRPFGEAPRELRLRATRAFVAARSFVQGLGVASD
VVRKVAQVPLGPECSRAVMKLVYCAHCLGVPGARPCPDYCRNVLKGCLANQADLDAEWRNLLDSMVLI
TDKFWGTSGVESVIGSVHTWLAEAINALQDNRDTLTAKVIQGCGNPKVNPQGPGPEEKRRRGKLAPRER
PPSGTLEKLVSEAKAQLRDVQDFWISLPGTLCSEKMALSTASDDRCWNGMARGRYLPEVMGDGLANQI
NNPEVEVDITKPDMTIRQQIMQLKIMTNRLRSAYNGNDVDFQDASDDGGSGHHHHHHHHHHGSGGLN
DIFEAQKIEWHEGSGYPYDVPDYA

Sequence information:

HUGO:	4449
Uniprot:	P35052
Refseq:	NM_002081.3

Structural information:

Topology:	Glycosylphosphatidylinositol (GPI) Anchored
PDB IDs:	4ACR;4AD7;4BWE;4YWT
Alphafold:	AF-P35052-F1

Expression profiles:

Human Protein Atlas	ENSG00000063660-GPC1

References

1. J. Trimmer. (2025). Anti-Glypican 1 (GPC1) [IPI-GPC1.25] in Immunohistochemistry (Rat). Addgene. https://doi.org/10.57733/addgene.7d1701

How to cite this antibody:
Anti-Glypican 1 (GPC1) [IPI-GPC1.25] - from Institute for Protein Innovation (IPI) (Addgene #240996; http://n2t.net/addgene:240996; RRID: AB_3697521).

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